REMARKS

Claims 1-26 are pending in the application.

I. Support for Claim Amendments

The claims were amended to more clearly define the invention and correct grammatical errors. Claim 1 was amended to recite that the method for forming matrix stabilized enzyme crystals resistant to degradation by proteolytic enzymes comprises the steps of contacting a crystalline enzyme with at least one polymer having one or more reactive moieties and cross-linking the reactive moieties on the polymer with a multi-functional cross-linking agent, effective to form a cross-linked, net-like polymer structure to adhere to the crystal layer of the crystalline enzyme. Support for these amendments is found in the Specification, for example, at the bottom of page 7 and at pages 8-9. No new matter is introduced by the amendments and entry thereof is respectfully requested.

II. Objection to the Specification

The disclosure is objected to because in the specification at page 3, line 2, the meaning of "polylyside" is allegedly uncertain. A "polylyside" polymer is used throughout the Specification interchangeably with "polylysine" to describe a polyamino acid with lysine as the reactive side chain moiety. See the Specification at, for example, the sentence spanning pages 7 and 8, and page 8, the first sentence of the first full paragraph. As the use of "polylyside" in the Specification is neither a misspelling nor the use of an uncertain term, Applicants respectfully request withdrawal of the objection to the disclosure.

III. Rejection of claims 1-26 under 35 U.S.C. § 112, first paragraph

Claims 1-26 are rejected under 35 U.S.C. § 112, first paragraph. Specifically, the Examiner rejected the claims because he alleges the Specification does not reasonably provide enablement for using other polymers and cross-linking agents other than those recited in claims 6 and 11. Further, he alleges that the results of using a polymer and cross-linking agent substantially different from those disclosed in the Specification would be unpredictable. Applicants respectfully traverse this rejection.

The claimed invention involves a method of forming matrix stabilized enzyme crystals by forming a cross-linked polymer matrix that adheres to the surface of the enzyme crystal. The polymer having one or more reactive moieties is used in conjunction with the cross-linking agent to form a net-like structure on the crystal surface. For example, the Specification at the bottom of page 8- page 9 discloses suitable polymers including cationic, anionic, and/or hydrophobic polymers that can include reactive moieties such as electrophilic and/or nucleophilic groups. When cross-linked with a bifunctional cross-linking agent, such as those described in the Specification at page 8, the reactive moieties form a net-like structure that adheres to the crystal surface.

Applicants submit that one of skill in the art would be able to use any polymer having reactive moieties capable of interacting with the surface of the crystal enzyme, including but not limited to the polymers recited in claim 11. One of skill in the art would know, from knowledge of the reactive moieties present on the polymer, which bifunctional cross-linking agents are suitable for use in conjunction with the polymer. Suitable cross-linking agents include, but are not limited to, those disclosed in claim 6. Applicants respectfully submit that it would not be

unpredictable as to results when using a polymer and cross-linking agent substantially different from those disclosed in the Specification because one of skill in the art would know from the Specification to select both a polymer with reactive moieties capable of interacting with the surface of the enzyme crystal and a suitable cross-linking agent for use in conjunction with the selected polymer. In view of the foregoing remarks, Applicants respectfully request that the rejection of claims 1-26 under 35 USC 112, first paragraph, be withdrawn.

IV. Rejection of claims 1-26 under 35 U.S.C. § 112, second paragraph

Claims 1-26 are rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner alleges that claims 1-26 are confusing and unclear by reciting "matrix stabilized enzyme crystals" since it is uncertain as to what constitutes the matrix and the physical and chemical relationship between the matrix and the enzyme crystals, and the polymer and cross-linking agent when required. Applicants respectfully traverse this rejection.

From the disclosure that Applicants have provided, one of skill in the art would understand that a "matrix stabilized enzyme crystal" refers to an enzyme crystal to which on its surface is adhered a net-like polymer matrix, comprising a polymer having reactive moieties cross-linked with a suitable cross-linking agent. The interaction between the polymer matrix and the enzyme crystals may be a chemical relationship, such as a charge-charge interaction, and/or a physical relationship, for example, wherein the polymer reactive moieties cross-link to each other and to external amino acid residues on the surface of the crystal. See the Specification at, for example, the bottom of page 8 to the top of page 9, and at the second paragraph of page 10.

Applicants respectfully disagree with the Examiner that claims 1-26 are confusing and unclear and that claims 1-20 are further confusing and unclear by claim 1 failing to set forth clear, distinct and positive process steps. Applicants have nevertheless amended claim 1 to recite that the method for forming matrix stabilized enzyme crystals resistant to degradation by proteolytic enzymes comprises the steps of contacting a crystalline enzyme with at least one polymer having one or more reactive moieties and cross-linking the reactive moieties on said polymer with a multi-functional cross-linking agent, effective to form a cross-linked, net-like polymer structure to adhere to the crystal layer of the crystalline enzyme.

Claim 21 was rejected because the recitation of the term "PAL" is allegedly uncertain as to the material required. Applicants have amended claim 21 to recite "phenylalanine ammonia lyase" instead of "PAL".

Claims 23 and 26 are allegedly confusing and unclear as to the invention claimed by not requiring both a polymer and cross-linking agent since the Specification describes the production of the matrix in the presence of a cross-linking agent and polymer. Applicants respectfully submit that the matrix stabilized enzyme crystals recited in claims 23 and 26 will be understood to one of skill in the art to be enzyme crystals to which on their surface is adhered a net-like polymer matrix, comprising a polymer having reactive moieties cross-linked with a suitable cross-linking agent. Applicants submit that in view of the description provided in the Specification, the separate recitation of a polymer and a cross-linking agent is not necessary in claims 23 and 26.

Claim 24 is allegedly confusing and unclear by reciting "cross-linking polylysine with phenylalanine ammonia lyase" since it is unclear how an enzyme can be used to cross-link a polymer. Applicants have amended claim 24 to recite that the crystals of phenylalanine

ammonia lyase are stabilized by polylysine cross-linked in the presence of less than 0.5% w/v bifunctional cross-linking agent.

In view of the foregoing remarks and amendments, Applicants respectfully request withdrawal of the rejection of claims 1-26 under 35 USC 112, second paragraph.

IV. Rejection of claims under 35 U.S.C. § 103(a)

Claims 1, 2, 4-8, 11-14, 18 and 19 are rejected under 35 USC 103(a) as being unpatentable over Margolin (6,541,606 B2)("Margolin '606") in view of Mucke (4,940,664). The Margolin '606 patent is relied upon by the Examiner for the disclosure of stabilizing enzyme protein crystals by encapsulating the protein crystals in a polymer, such as polyamino acids, polyesters, or polyols. Margolin '606 further discloses the protein crystal may be cross-linked with a multifunctional cross-linking agent, the amount of which can be 1.5%; the protein crystal may be an enzyme crystal; the enzyme crystal may be cross-linked; and the rate of dissolution of the polymer encapsulated protein crystals modulated by varying polymer cross-linking. Mucke is relied upon for the disclosure that carrier-bound enzymes may be stabilized by treatment with a bifunctional cross-linking agent such as glutaraldehyde and a polyamine such as polyethylene imine. The enzyme is bound to an inorganic carrier and then cross-linked with glutaraldehyde to produce a carrier bound enzyme, which is then treated with glutaraldehyde and polyethylene imine. The Examiner alleges it would have obvious when producing a polymer encapsulated enzyme crystal as taught by Margolin '606, to use a polyamino acid as the polymer as taught by Margolin '606, and it would have been further obvious to use a glutaraldehyde to cross-link the polyamino acid as taught by Mucke. Since Margolin '606 discloses using 1.5% cross-linking

agent to cross-link enzyme crystals, the Examiner alleges it would have been obvious to use this amount of cross-linking agent in combination with the polyamino acid polymer.

Applicants respectfully traverse the Examiner's rejection of the claims as being obvious over Margolin '606 in view of Mucke. Margolin '606 teaches the encapsulation of protein crystals in polymeric carriers for the stabilization, storage and delivery of biologically active macromolecules, such as protein crystals. The Examiner points to the Margolin '606 patent disclosure of polymers used for encapsulation of protein crystals, in the paragraph bridging cols 12 and 13 and the paragraph bridging cols 28 and 29. However, in the disclosure preceding the paragraph bridging columns 28 and 29, Margolin '606 teaches how the polymer encapsulated protein crystals are formed. At col. 27, line 45, the process of protein encapsulation is described as starting with suspending protein crystals in a polymeric carrier dissolved in an organic solvent. In the paragraph bridging columns 27 and 28, Margolin '606 discloses that after this contact between the protein crystals and the dissolved polymeric carrier, the crystals become coated and are referred to as nascent microspheres. The suspended coated crystals or microspheres are then transferred along with the polymeric carrier and organic solvent to an aqueous solution containing an emulsifier. While the polymer-coated crystals are immersed in the aqueous phase, the organic solvent evaporates or diffuses away from the polymer. Eventually, the polymer precipitates, forming a solid phase that encapsulates the protein crystal.

What Applicants are teaching is a method of forming matrix stabilized enzyme crystals by forming a cross-linked polymer matrix that adheres to the surface of the enzyme crystal. The polymer having one or more reactive moieties is used in conjunction with the cross-linking agent to form a net-like structure on the crystal surface. The interaction between the polymer matrix and the enzyme crystals may be a chemical relationship, such as a charge-charge interaction,

and/or a physical relationship, for example, wherein the polymer reactive moieties cross-link to each other and to external amino acid residues on the surface of the crystal. In order for a combination of references to render the instant invention obvious, all the limitations of the invention need to be taught or suggested. Margolin '606 does not teach or suggest cross-linking the reactive moieties on the polymer with a multi-functional cross-linking agent, effective to form a cross-linked, net-like polymer structure to adhere to the crystal layer of the crystalline enzyme. Further, the polymer encapsulated protein crystals of Margolin are formed by phase separation techniques and do not involve a polymer matrix in contact with an enzyme crystal based on charge-charge interactions and/or cross-linking reactions. The Mucke reference teaches inorganic carrier bound enzymes cross-linked with a bifunctional cross-linking agent and a polyamine. However, this teaching does not cure the deficiencies of the Margolin '606 patent, which fails to describe the claimed cross-linked net-like polymer structure.

Applicants have amended claim 1 to recite that the "cross-linked, net-like polymer structure" is formed in accordance with the present invention to further obviate the rejection over Margolin '606 and Mucke. In view of the foregoing arguments, Applicants respectfully request that the rejection of claims 1, 2, 4-8, 11-14, 18 and 19 under 35 USC 103(a) over Margolin '606 in view of Mucke (4,940,664) be withdrawn.

Claims 9, 10, 16, and 17 are rejected under 35 USC 103(a) as being unpatentable over Margolin '606 in view of Mucke and Margolin (U.S. Patent No. 6,140,475)("Margolin '475"). The Margolin '475 patent is relied upon by the Examiner for the disclosure of cross-linking enzyme crystals using an amount of glutaraldehyde in a range of about 0.1 to about 0.2%. From the foregoing remarks, the combination of Margolin '606 and Mucke does not teach or suggest cross-linking the reactive moieties on the polymer with a multi-functional cross-linking agent,

effective to form a cross-linked, net-like polymer structure to adhere to the crystal layer of the crystalline enzyme, as claimed herein. The disclosure of the Margolin '475 patent does not cure these deficiencies. Margolin '475 does not depart from the disclosure of the Margolin '606 patent to address the claim-designated cross-linked net-like polymer structure. Accordingly, Applicants respectfully request that the rejection of claims 9, 10, 16, and 17 under 35 USC 103(a) over Margolin '606 in view of Mucke and further in view of Margolin '475 be withdrawn.

Claims 3, 15, 20-23 and 26 are rejected under 35 USC 103(a) as being unpatentable over Margolin '606 in view of Mucke and Eigtved (U.S. Patent No. 5,753,487). Eigtved is relied upon by the Examiner for the disclosure of stabilizing phenylalanine ammonia lyase by cross-linking with glutaraldehyde, and using the cross-linked phenylalanine ammonia lyase to treat hyperphenylalaninemia. From the foregoing remarks, the combination of Margolin '606 and Mucke does not teach or suggest cross-linking the reactive moieties on the polymer with a multi-functional cross-linking agent, effective to form a cross-linked, net-like polymer structure to adhere to the crystal layer of the crystalline enzyme. The disclosure of Eigtved ('487) does not cure this deficiency and teach a cross-linked, net-like polymer structure as claimed. Applicants respectfully request that the rejection of claims 3, 15, 20-23 and 26 under 35 USC 103(a) over Margolin '606 in view of Mucke and further in view of Eigtved be withdrawn.

Finally, claims 24 and 25 are rejected under 35 USC 103(a) as being unpatentable over Margolin '606 in view of Mucke, Eigtved, and Margolin '475. Eigtved is relied upon by the Examiner for the disclosure of stabilizing phenylalanine ammonia lyase by cross-linking with glutaraldehyde, and using the cross-linked phenylalanine ammonia lyase to treat hyperphenylalaninemia. Margolin '475 is relied upon by the Examiner for the disclosure of